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Habitat-specific differences in thermal plasticity in natural populations of a soil arthropod.

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Abstract

When populations experience substantial variation in environmental conditions, they may evolve phenotypic plasticity in response to these varying selection pressures. Evolutionary theory predicts differentiation in the level of phenotypic plasticity among different habitats. We evaluated temperature-induced phenotypic responses in juvenile growth rate in natural populations of the springtail *Orchesella cincta*, inhabiting forest and heathland. These habitats typically co-occur but differ strongly in e.g. thermal regime, relative humidity and structure. Offspring of females from the two habitats were reared at different temperatures in climate rooms and temperature response of juvenile growth rate and egg size was measured. We found a habitat-specific difference in plasticity of juvenile growth rate. The reaction norms of the forest populations were steeper than the reaction norms for heath populations at two replicated sampling sites. Egg weight itself demonstrated to be a plastic trait with higher egg weight at low temperatures, but the thermal response did not differ between habitats. We conclude that these populations have diverged evolutionary due to strong local selection. Our results support the argument that the level of phenotypic plasticity itself can be under selection and that differentiation in reaction norms can occur even in neighbouring habitats with no barrier to gene flow.

Keywords: juvenile growth rate, *Orchesella cintca*, phenotypic plasticity, reaction norm, temperature, parasympatric, divergent selection, litter.

Introduction

All natural environments show substantial variation in important abiotic conditions such as temperature, light and humidity. To maximize their fitness, organisms should be able to respond to these varying selection pressures. If fluctuations in environmental conditions occur at a time scale shorter than the generation time, selection is expected to favour phenotypic plasticity (Padilla & Adolph, 1996; Kingsolver & Huey, 1998). A whole body of theory has examined the conditions under which phenotypic plasticity is predicted to be favoured, such as habitat heterogeneity and the colonization of new habitats (Agrawal, 2001; Yeh & Price, 2004; Richards et al., 2006). Empirical field studies on morphology of butterflies indeed showed phenotypic plasticity to be favoured in habitats with seasonal heterogeneity (Brakefield *et al.*, 1996; Larsen, 1996).

Moreover, theory also predicts the level of phenotypic plasticity to be subject to selection (Gilchrist, 1995; Via *et al.*, 1995; De Jong, 2005). The level of phenotypic plasticity is defined as the degree to which a trait value changes in response to a change in the environment. Indeed, in habitats that are relatively stable with respect to one condition, individuals are found to have a lower level of plasticity in the trait affected than individuals in more variable environments (Ellers & van Alphen, 1997; Pfennig & Murphy, 2002; Price, Qvarnström & Irwin, 2003). For example, high variation in food conditions is correlated with high levels of plasticity in reproductive allocation in woodlice (Hassall *et al.*, 2005).

Regardless of considering fixed or plastic traits, local adaptation can only lead to genetic differences between populations if natural selection is stronger than homogenizing gene

flow. Over the past years, it has become accepted that genetic divergence is possible without high levels of isolation, i.e. in sympatric or parapatric populations (Schneider *et al.*, 1999; Schilthuizen, 2000; Luttikhuizen *et al.*, 2003). However, population divergence in phenotypic plasticity has only been studied occasionally and these studies have been mainly restricted to geographically isolated (allopatric) populations (Heschel *et al.*, 2004; Hassall *et al.*, 2005; Lehmann & Rebele, 2005). It is therefore unresolved if natural selection on phenotypic plasticity is strong enough to maintain differences in the presence of gene flow.

Here we investigate differences in phenotypic plasticity in a soil arthropod, *Orchesella cincta*. We considered forest and heath, two habitats that differ e.g. in the amplitude of daily temperature fluctuations, relative humidity and structure, but are also naturally co-occurring. Recent studies based on microsatellite markers (van der Wurff *et al.*, 2005), mtDNA and AFLP markers (van der Wurff *et al.*, 2003; Timmermans *et al.*, 2005) suggest some isolation by distance between *O. cincta* populations in NW Europe, but over distances smaller than 60 km the species is genetically homogeneous as a result of gene flow (van der Wurff *et al.*, 2003). Divergence in the level of phenotypic plasticity between the habitats can therefore not be explained by genetic drift but must be the result of selective forces.

We measured differences in plasticity of juvenile growth rate of *O. cincta* from these two habitats in response to temperature. This species of springtail lives in the litter layer and is therefore dependent on soil temperature for its development. As development time and hence growth rate are highly correlated with survival rate, we expect growth rate to be an important fitness characteristic of juvenile *O. cincta*. Differences in phenotypic plasticity

can be quantified by measuring a reaction norm (Via *et al.*, 1995; Roff, 1997). A thermal reaction norm describes a phenotypic value under different temperature conditions, of which the slope indicates the level of plasticity, i.e. a steeper line is considered to be more plastic (De Jong, 1990). As we hypothesise to find more plastic populations in more variable habitats, we expect the reaction norms for growth to be steeper in heath habitats than in forest habitats.

Materials and methods

Orchesella cincta (Collembola) is a species of springtail found in the litter layer in a broad range of habitats in the Holarctic. The species can reach very high local densities, (van Straalen, Verhoef & Joosse, 1985), and is found in abundance in a wide variety of forests and woodlands (personal observation). Like all springtail species, *O. cincta* grows indeterminately, with moults separating instars. Animals used in the experiments were collected in May 2004 from the litter layer in forest and heath in both the Kampina (51˚34' N, 5˚15' E) and the Bussumer Heide (52˚15' N, 5˚10' E), two nature reserves in The Netherlands. The two sites contain large areas of heathland surrounded by mixed forest on a sandy soil. Both heath and forest habitats were sampled at three locations (maximally 500 m apart). This was done at both sites, resulting in 12 (sub)samples. *Orchesella cincta* is found throughout the heath but as densities in open heath vegetation were rather low, samples taken from the heathland were always in the vicinity of a solitary tree. The differences in temperature and relative air humidity were recorded every two hours with stand-alone Micrologs (Mini Logger EC650, ±0.6˚C, RH ±3%) in both forest and heath in the Kampina from August till October in 2005. Dataloggers were

106 placed in the litter layer, up to 10 cm under the leaf litter layer on the soil surface. For the
107 12 sampling locations, a stock population was set up with 25-30 individuals in a climate
108 room (20˚C, 70% RH, LD 12:12 h). All populations were fed algae on bark
109 (*Desmococcus* spec). Ten random individuals of each sampling location were placed in Ø
110 7 cm pots with a bottom of plaster of Paris with a continuous supply of fresh food to
111 produce offspring for the first experiment. These pots were checked for eggs daily.
112 Offspring in one egg batch are always sired by one male only (Gols, Ernsting & van
113 Straalen, 2004). Egg batches, i.e. clusters of full sibling eggs, were divided into three
114 groups; one to measure egg dry weight and two to measure juvenile growth rate at two
115 temperatures. Approximately 30-50 freshly laid eggs were collected in a silverfoil cup
116 containing a drop of 95% ethanol. The ethanol was allowed to evaporate after which a
117 tray of cups was placed in a desiccation tank with silica gel for at least 10 days for the
118 eggs to be dried completely.
119 The remaining part of the egg batch was randomly divided over two series of pots at two
120 temperatures (12˚C and 20˚C at 70% RH, LD 12:12 h) so full siblings were reared at both
121 temperatures. Eggs were placed in Ø 2.5 cm pots with a bottom of plaster of Paris.
122 Depending on the original size of the egg batch, 10 to 40 eggs were placed per pot. At
123 20˚C juveniles were freeze-dried approximately 19 days after hatching and at 12˚C after
124 approximately 40 days in order to end up with a comparable weight. As estimated from
125 mean adult weight (Ernsting & Isaaks, 2002) and growth rates at several temperatures
126 (Driessen, Ellers & van Straalen, 2007), juveniles at this age were not fertile yet and no
127 eggs of spermatophores were observed. After the period of growth, 10 individuals were
128 chosen randomly to be freeze-dried. From the final dry weight measurements, juvenile

129 growth rate, (ln dry weight) · t^{-1}, could be calculated. Juvenile growth rate has a linear
130 relation with temperature making interpolation between data points possible (Janssen &
131 Joosse, 1987). Egg weight was neglected as this is a small fraction of the total juvenile
132 dry weight and because egg weight did not differ between the different populations (see
133 results).
134
135 A second experiment was carried out in addition to the measurements on egg weight
136 described above in order to compare the thermal response of egg weight between
137 habitats. For this purpose we used animals reared in the lab from the outbreeding stock
138 population that was started with animals from the field. This population had been kept in
139 the climate room for at least two generations to exclude maternal effects. These animals
140 were allowed to lay eggs both at 16˚C and 20˚C after a three week acclimation period.
141 Although eggs hatch and juveniles grow and reach maturity at 12˚C, female reproduction
142 was very low at this temperature, hence we chose to do the experiment at 16˚C. Again,
143 egg dry mass was determined and compared over the two temperatures.
144
145 Data on juvenile growth rate were analyzed with a mixed linear model in SAS 9.1. This
146 analysis can handle unbalanced data with both fixed and random factors better than a
147 double nested ANOVA (Verbeke & Molenberghs, 1997; Littell *et al.*, 2006), though
148 results were similar (data not presented). Data on egg dry weight were not unbalanced
149 and were therefore analysed with the general linear model procedure in SPSS 11.5.
150
151 Results

Temperature regimes of habitats.

As an indication for the extent to which the habitats differ, temperature fluctuations for forest and heath are summarized in Table 1. Although mean temperature did not differ between the habitats, the amplitude of temperature fluctuations is much higher for heathland.

Temperature-induced plasticity in juvenile growth rate.

As expected, juvenile growth rate showed a clear plastic response to temperature, with all juveniles growing faster at higher temperatures (Table 2). Habitat had a small but significant effect on juvenile growth rate. In general, individuals from forest populations grew faster than individuals from heath, except for the Bussumer Heide population at 12˚C. An interaction between habitat and temperature was found for juvenile growth rate, with the effect of temperature being stronger in forest individuals (Fig.1). This may seem a small difference in growth rate, but it can result in an animal from a forest habitat reaching maturity approximately 3 days earlier on a total of 23 days (a 13% reduction) at 20˚C than an animal from heathland. This effect is stronger at lower temperatures where growth rates are lower. The heathland population at the Bussumer Heide for example, will reach maturity at 12˚C approximately 8 days earlier on a total of 45 days (an 18 % reduction) in comparison to the forest population. There is an interaction between site and temperature, the effect of temperature being stronger for Kampina. Batch (cluster of full-sibling eggs) had a significant effect indicating that variation between siblings was smaller than between non-siblings and confirms a genetic component. No correction for number of eggs per pot was needed as no density dependent effects were observed.

175

176 *Dry weight of eggs.*

177 There was no difference in dry weight of eggs laid at 20˚C between the different habitats
178 ($F_{1, 54}$=0.366, P=0.55). Therefore differences in plasticity of juvenile growth rate cannot
179 be explained by differences in egg size. There was also no interaction between habitat
180 and site ($F_{1, 54}$=1.655, P=0.204).
181 Data gained from the second experiment in which eggs were laid at 16˚C and 20˚C
182 showed a strong response to laying temperature (Table 3). Eggs laid at a lower
183 temperature were approximately 25% heavier. However, the level of temperature-induced
184 plasticity did not differ between habitats. A small but significant effect of site was
185 detected, with a higher average weight for eggs from Kampina (Fig. 2).

186

187 Discussion.

188 When populations experience differences in the magnitude of environmental variation,
189 they may evolve different plasticity levels to maximize fitness under these varying
190 selection pressures. In this study, we found a specific difference in phenotypic plasticity:
191 the reaction norms of the forest populations were steeper than the reaction norms for
192 heath populations for two replicated sample sites. This is in contrast to our expectation as
193 we find the heath habitat to be more variable than forest habitat, and highly variable
194 environments are thought to select for strong plasticity. Until now most work has been
195 done on morphological and physiological traits and not on fitness traits. Therefore,
196 hypotheses formulated from this background may not be applicable to work on fitness
197 traits. Richards *et al.* (2006) point out the difference between plasticity in fitness and non-

198 fitness traits. Plasticity in non-fitness traits can be adaptive if either morph is favoured in
199 a different environment. In contrast, natural selection will select for high fitness levels in
200 each environment and therefore low plasticity in a fitness trait like survival or growth
201 rate. Achieving such low plasticity of a fitness trait over different environments may very
202 well depend upon high plasticity in underlying morphological or physiological traits
203 (Richards *et al.*, 2006). Although the consensus on phenotypic plasticity is that more
204 variable environments select for higher plasticity in morphological or physiological traits,
205 this may very well be the opposite when regarding life-history traits. Alternatively,
206 differentiation in plasticity might be a side-effect of selection on a different trait, for
207 instance cold adaptation (Birkemoe & Leinaas, 2001). There is yet no consensus as to
208 how the steepness of the reaction norm is related to performance at more extreme
209 temperatures (van Straalen & van Diepen, 1995; Trudgill *et al.*, 2005).
210 Our results showed no difference between habitats in the response of egg size to
211 temperature. In both habitats egg weight was higher at lower temperatures as is found in
212 many insects, e.g. Lepidoptera (Fischer, Brakefield & Zwaan, 2003), Diptera (Avelar,
213 1993; Huey *et al.*, 1995) and Coleoptera (Ernsting & Isaaks, 1997). Investment in egg
214 size is a known maternal effect influencing development and growth rate of offspring
215 (Fox *et al.*, 1999). Since dry weight of the eggs laid at 20˚C did not differ between the
216 different habitats, we conclude that differences in the plasticity of growth rate between
217 habitats are not caused by confounding maternal effects of egg size.
218 In this discussion, considering the costs of plasticity is just as important as understanding
219 the possible benefits. If costs of maintaining plasticity are high (DeWitt, Sih & Wilson,
220 1998; Edelaar, Piersma & Postma, 2005), selection should not only be quick to remove it

in an environment where it is of little use, but also from an environment that poses strong demands on resource allocation other than maintaining plasticity levels. However, at present the costs of thermal plasticity are poorly understood.

Comparing the norms of reaction between two replicate sites demonstrates that, with respect to temperature, the populations from the more variable heathland have a flatter reaction norm for a life history trait like juvenile growth than the populations from the more stable forest habitats. These typical environment-dependent phenotypic differences between populations can have important consequences for local adaptation and habitat-specific differentiation. The present study shows that differences in habitats over a small range can maintain different levels of phenotypic plasticity, even over a distance of 500 m with free gene flow. Our research also strongly supports the argument that the level of plasticity itself can be under direct selection (Via *et al.*, 1995), and that plasticity in life history traits may follow a different trend than expected from previous work.

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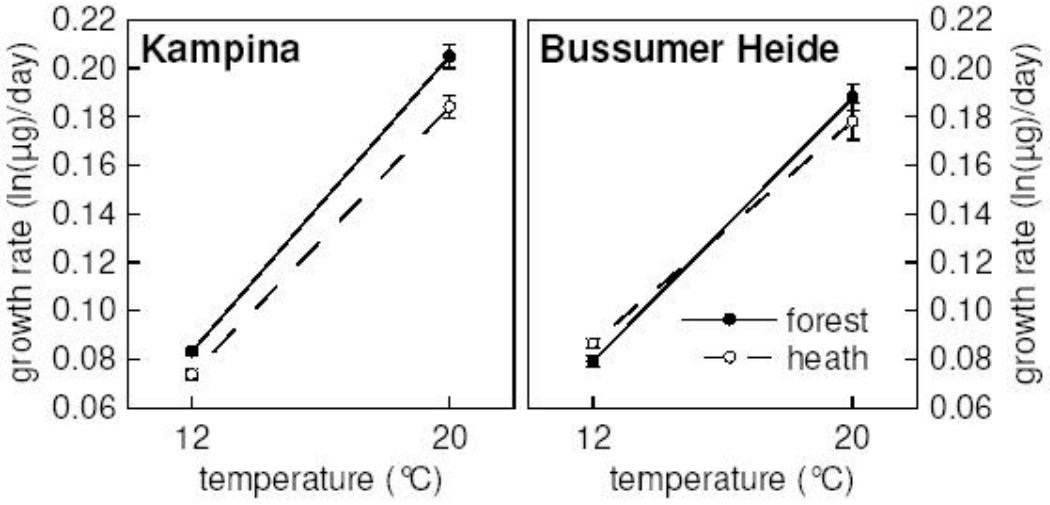
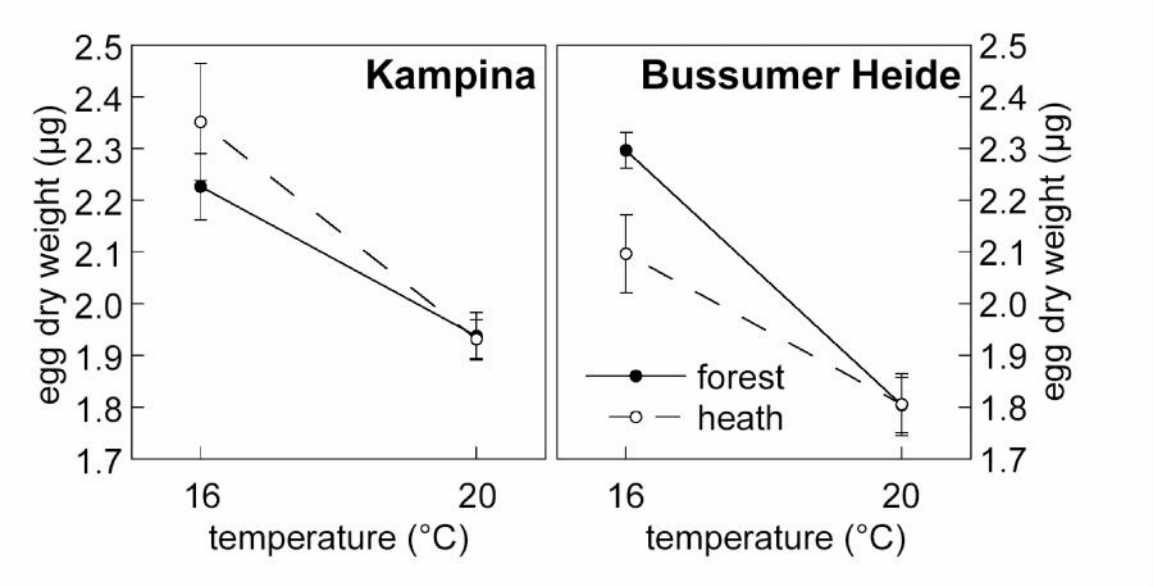


354

355 Fig 1. Reaction norms of juvenile growth rate (mean ±1SE) in relation to temperature for
356 the two habitats (forest and heath) from the Bussumer Heide and Kampina. In the
357 Bussumer Heide populations the heath population has a higher growth rate at 12˚C, while
358 the forest population has a higher growth rate at 20˚C. In the Kampina populations, the
359 growth rate of the forest population exceeds the growth rate of the heath population over
360 this part of the temperature range. In both graphs, the reaction norm for growth rate is
361 steeper in the forest population.

362



363

364

365 Fig. 2. Egg dry weight (mean ±1SE) for eggs laid at 16˚C or 20˚C compared between the
366 two habitats (forest and heath) for Bussumer Heide and Kampina. Temperature itself has
367 a strong effect on egg weight, but no difference in response was found between the
368 habitats per site.

369

Table 1. Differences in temperature as measured in the litter layer summarized for the two habitats (forest and heath) over the period 30 August - 10 October 2005. Temperatures (°C) were measured 10 cm in the litter layer every two hours. Mean temperature gives the overall mean measured over all days. While mean temperature did not differ (Paired T-test; $t = -1.68$, $P > 0.5$), daily variation in temperature is higher in heath than in forest with higher (Paired T-test; $t = 4.52$, $P < 0.001$) and lower (Paired T-test; $t = -5.63$, $P < 0.001$) daily temperatures. The mean SD is calculated by taking the mean over all the SD's per day and was also found to be significantly different between the two habitats (Paired T-test; $t = 6.88$, $P < 0.001$).

Habitat	mean daily maximal T (°C)	mean daily minimal T (°C)	mean T (°C)	mean daily SD
Heath	20.04 (SD 4.21)	10.25 (SD 3.39)	14.11 (SD 3.32)	3.25 (SD 1.34)
Forest	18.46 (SD 3.42)	11.44 (SD 2.80)	14.39 (SD 2.64)	2.38 (SD 1.12)

Table 2. Results of a full factorial linear mixed model on juvenile growth rate for the effects of batch (egg batch split over the two temperatures), site (nature reserve, Kampina or Bussumer Heide), habitat (forest or heath) and temperature (12˚C or 20˚C). Wald statistic value for random effects significant (*** P<0.001 and * P<0.05).

Random effect				
Covariance Parameter		Estimates	SE	Wald Z
Batch		0.000167	0.000012	4.624 ***
Residual		0.000245	0.000036	19.993 ***
Fixed effect				
Effect	Df	Den Df	F value	P
Site	1	51	0.80	0.3758
Habitat	1	51	5.26	0.0260 *
Temperature	1	798	7374.18	<0.001 ***
Site x habitat	1	51	2.22	0.1427
Habitat x temperature	1	798	34.84	<0.001 ***
Site x temperature	1	798	28.03	<0.001 ***
Site x habitat x temp	1	798	1.78	0.1819

Table 3. General linear model with dependent variable egg dry weight (µg) for the effects of site (nature reserve Kampina or Bussumer Heide), habitat (forest or heath) and temperature (16˚C or 20˚C). Eggs were laid at the two temperatures after which egg dry weight was measured. Temperature had the strongest effect on egg weight, as well as a minor effect of site.

Factor	Df	Mean Square	F	P
Site	1	0.165	4.082	0.048 *
Habitat	1	0.005	0.131	0.719
Temperature	1	1.864	46.253	<0.001 ***
Site x habitat	1	0.085	2.106	0.152
Site x temp	1	0.005	0.114	0.737
Habitat x temperature	1	0.004	0.101	0.751
Site x hab x temp	1	0.091	2.268	0.137
Error	64	0.040		